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1. Sier et al, Gastroenterology, 1994, 107:1449-1456)

2.Ganesh et al, Cancer Res., 1994, 54:4065-4071).

3. Janicke et al, Sem. Throm. Hemostasis, 1991, 17:303-312)

4. Nekarda et al (Cancer Res., 1994, 54:2900-2907)

5. Grandahl-Hansen et al, 1993, Cancer Research 53:1513-1521)

6. JOURNAL OF.NEURO-ONCOLOGY, (1994) Vol. 22, No. 2, pp. 139-151.

7. INTERNATIONAL JOURNAL OF ONCOLOGY, (MAR 1994) Vol. 4, No. 3, pp. 717-721.

8. Biol.Chem.Hoppe Seyler (376, No. 5, 259-67, 1995) 2 Fig. 67 Ref.

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[CANCER RESEARCH 54, 2900–2907, June 1, 1994]

Prognostic Impact of Urokinase-type Plasminogen Activator and Its Inhibitor PAI-1 in Completely Resected Gastric Cancer[1]

Hjalmar Nekarda,[2] Manfred Schmitt, Kurt Ulm, Andrea Wenninger, Holger Vogelsang, Karen Becker, Jürgen D. Roder, Ulrich Fink, and Jörg R. Siewert

Chirurgische Klinik und Poliklinik [H. N., A. W., H. V., J. D. R., U. F., J. R. S.]; Frauenklinik [M. S.]; Institut für Medizinische Statistik und Epidemiologie [K. U.]; and Institut für Allgemeine Pathologie und Pathologische Anatomie [K. B.], Technische Universität München Ismaningerstr. 22, D-81675 München, Germany

ABSTRACT

The prognostic impact of the proteolytic factors urokinase-type plasminogen activator (uPA) and plasminogen activator inhibitor type 1 (PAI-1) was evaluated in 76 completely resected gastric cancer patients enrolled in a prospective study. All patients underwent macroscopically and microscopically residual tumor-free resection (category R_0, Union International Contre Cancer, 1987). uPA and PAI-1 levels were quantified in detergent-extracted (Triton X-100) specimens of primary gastric tumors by enzyme-linked immmunosorbent assays. Median values of 1.57 ng uPA/mg protein were determined in tumor tissue extracts compared to 0.14 ng uPA/mg protein in normal mucosa. For PAI-1, 0.93 ng PAI-1/mg protein *versus* 0.09 ng PAI-1/mg protein was calculated. uPA levels in tumor tissue extracts were significantly correlated with vascular invasion, Laurén classification, and WHO classification, whereas PAI-1 levels showed a significant correlation with advanced lymph node involvement, depth of invasion, tumor stage, site of tumor, and the Laurén, Borrmann, and WHO classifications.

Elevated uPA and PAI-1 levels were found to be associated with poor prognosis. The optimal cutoff values indicating a group of patients with shorter survival were 1.5 ng uPA/mg protein and 1.25 ng PAI-1/mg protein, respectively (Classification and Regression Tree analysis). Patients with either high uPA or PAI-1 values were significantly associated with decreased survival (median time of survival was 23 months (high) *versus* 44 months (low). By univariate Cox regression analysis, it was shown that TNM categories, WHO classification, size of tumor, uPA and PAI-1 levels were all significantly associated with survival. However, in multivariate Cox regression analysis of these grouped variables, nodal status, PAI-1 levels, and WHO classification were the only independent prognostic factors. The relative risks of failure were 5-, 2.9-, and 2.4-fold, respectively. We conclude that PAI-1 and uPA positivity may serve as new prognostic factors in gastric cancer, predicting shorter survival even in clinically important subgroups of patients.

INTRODUCTION

Tumor-associated proteases (*e.g.*, plasmin, uPA,[3] cathepsins, and metalloproteases) and their inhibitors are important factors involved in tumor invasion and metastasis (1–6). In breast cancer, proteolytic factors such as cathepsin D, uPA, and its inhibitor PAI-1 have been claimed to be of independent prognostic value for disease-free and overall survival in several independent studies (7–13). In gastrointestinal cancer, elevated uPA and PAI-1 levels in the primary tumor have been described for esophageal cancer (14, 15), gastric cancer (15–20), and colorectal cancer (18, 21–24) when compared with normal mucosa. For gastric cancer patients, positive correlations of histomorphological data with uPA and PAI-1 levels have been described (17, 19), but to our knowledge the clinical significance of these findings for survival has not yet been demonstrated. We report on a prospective study describing the independent prognostic impact of both uPA and PAI-1 for survival in patients with gastric cancer who have been resected without any indication of residual tumor (category R_0, UICC, 1987).

MATERIALS AND METHODS

Patients. Seventy-six patients with gastric cancer (28 women: median age, 63; range, 34–83 years; and 48 men: median age, 64; range, 31–79 years) were consecutively enrolled into a prospective study between April 1987 and December 1989. Inclusion criteria for patients studied were absence of distant or peritoneal metastases and a macroscopically and histologically complete surgical removal of the primary gastric cancer (category R_0, UICC, 1987; Ref. 25), survival time > 90 days, and a complete follow-up.

Operation. Surgery was performed at the Department of Surgery, Klinikum rechts der Isar, Technical University of Munich. Gastric resection and lymphadenectomy were carried out according to the standardized protocol of the German Gastric Cancer Study Group (26, 27). The lymph nodes of compartments I and II (28) were completely removed by "en bloc" dissection. As a matter of routine, the lymph nodes of the ligamentum hepatoduodenale (station 12) and the retroduodenal lymph nodes (station 13) of compartment III were dissected in patients with advanced cancer (pT > 1) (29). In 35 patients (46%), the tumor was located in the proximal third of the stomach. Nine of these patients were resected by total gastrectomy, 15 by transhiatal or left regional extended gastrectomy, 7 by transmediastinal esophago-fundectomy, and 4 by esophago-gastrectomy. In 12 patients (16%), cancer was located in the middle third, and in 22 patients (29%), in the distal third of the stomach. Twenty-three of these patients were resected by total gastrectomy, 2 by left regional extended gastrectomy, and 9 by subtotal gastric resection. In 7 patients (9%), the entire stomach was affected by carcinoma. Five of these patients were resected by total gastrectomy and 2 by left regional extended gastrectomy.

Histomorphological Examination. The examination followed the protocol of the German Gastric Cancer Study Group (26). Twenty-three patients (30%) were classified as stage I (IA, $n = 6$, 8%; IB, $n = 17$, 22%), 13 patients (17%) were classified as stage II, 28 patients (37%) were classified as stage III (IIIA, $n = 18$, 13%; IIIB, $n = 10$, 13%), and 12 patients (16%) were classified as stage IV. Several histomorphological parameters with prognostic relevance were investigated: exclusion of residual tumor at the resection margins of primary tumor and of the "en bloc" lymph node resectum (category R, UICC, 1987); Borrman classification (30); diameter and site of tumor; typing and grading according to the WHO classification (31); Laurén classification (32); presence of lymphangiosis carcinomatosa; vascular invasion; and perineural carcinosis. The number of involved lymph nodes, the percentage of involved lymph nodes, and involvement of lymph node compartments I, II, and III (stations 12 and 13, according to the Japanese Research Society of Gastric Cancer; Ref. 28) were investigated in the adherent lymph drainage tissue. For survival analysis, the WHO classification was categorized into "differentiated" or "undifferentiated" type according to the report by Sugano *et al.* (33).

Follow-up. Adjuvant chemotherapy was not administered. Thirty-eight patients (50%) died because of tumor recurrence; the median time of survival of these patients was 20 months (range, 4–51 months). At the end of the observation period, 32 patients (42%) were still alive; their median follow-up was 30 months (range, 19–52 months). One living patient showed a local relapse. Six patients (8%) died due to other causes (four of cardiovascular

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[2] To whom requests for reprints should be addressed, at Chirurgische Klinik der Technischen Universität München, Klinikum rechts der Isar, Ismaningerstr. 22, D-81675 München, Germany.

[3] The abbreviations used are: uPA, urokinase-type plasminogen activator; PAI-1, plasminogen activator inhibitor type 1; UICC, Union International Contre Cancer; ELISA, enzyme-linked immunosorbent assay; CART, classification and regression tree(s); RR, relative risk.

disease, one of iatrogenic complication, and one of nephrotic syndrome). For survival analysis, however, these patients were treated as censored observations. The median survival time (34) calculated for all patients was 37 months.

Tissue Extraction and ELISA for uPA and PAI-1. Immediately after resection, fresh tumor specimens and normal mucosa (both about 1 cm^3) were selected, snap-frozen, and stored in liquid nitrogen. Cryostat sections (5-μm thickness) were prepared from the tumor specimens and the normal mucosa, which were then stained with hematoxylin and eosin to confirm presence (tumor) or absence (normal mucosa) of tumor cells. Subsequently, 20 to 30 sections of 60 μm each (total wet tissue weight between 70 and 100 mg) were cut for tissue extraction. The last section (5-μm thickness) was also stained by hematoxylin and eosin to demonstrate presence or absence of tumor cells. The still frozen cryostat sections were dipped into liquid nitrogen and then pulverized in a Micro-Dismembrator from Braun-Melsungen (Melsungen, Germany). The still frozen powder was dispesed with 400 μl TBS (0.02 M Tris-HCl-0.125 M NaCl, pH 8.5) containing 1% nonionic detergent Triton X-100 (Sigma, Munich, Germany). The suspension was gently shaken over night at 4°C and then subjected to ultracentrifugation (100,000 × g for 45 min at 4°C) to separate tissue debris. Supernatants were collected, divided into 50-μl aliquots, and stored in liquid nitrogen until use. Levels of uPA and its inhibitor PAI-1 in the tissue extracts were determined by commercially available ELISA kits (American Diagnostica, Inc., Greenwich, CT) for uPA and PAI-1. Details of the kits are described elsewhere (10, 11, 35). The lower limit of detection is 10 pg/ml for uPA and 40 pg/ml for PAI-1. Levels of uPA and PAI-1 are expressed in ng/mg protein. Protein content of the tissue extracts was determined by the Pierce BCA protein assay kit (Pierce, Rockford, IL; Ref. 36).

Statistics. Statistical analysis was performed using the BMDP software package (BMDP Statistical Software, Inc., Los Angeles, CA; Ref. 37). Differences in uPA or PAI-1 levels and histomorphological variables among various groups of patients were analyzed by the Kruskal-Wallis test and the Mann-Whitney two-sample test. All tests were performed at a significance level of $P \leq 0.05$. To determine the relative prognostic impact of uPA and PAI-1 compared to established prognostic factors (histomorphological variables) in a prospective fashion, survival was analyzed according to Cox's proportional hazard model (38). Statistical analyses included continuous as well as binary covariates, all of which were considered as fixed (not time-dependent). For Cox regression, continuous covariates were examined to determine whether the respective failure rate showed an exponential development due to the assumptions of the Cox model. If not, the CART (39–41) technique was used to determine the optimal cutoff value to recode the continuous variable into a binary one. Briefly, CART takes the value with maximal log-rank test for discrimination of high and low levels. The 95% confidence interval for these cutoff points was calculated by a test-based method. Group-oriented curves for survival were calculated according to the Kaplan-Meier (34) model. The relative risks of the various prognostic variables and the corresponding 95% confidence intervals were estimated according to the Cox model.

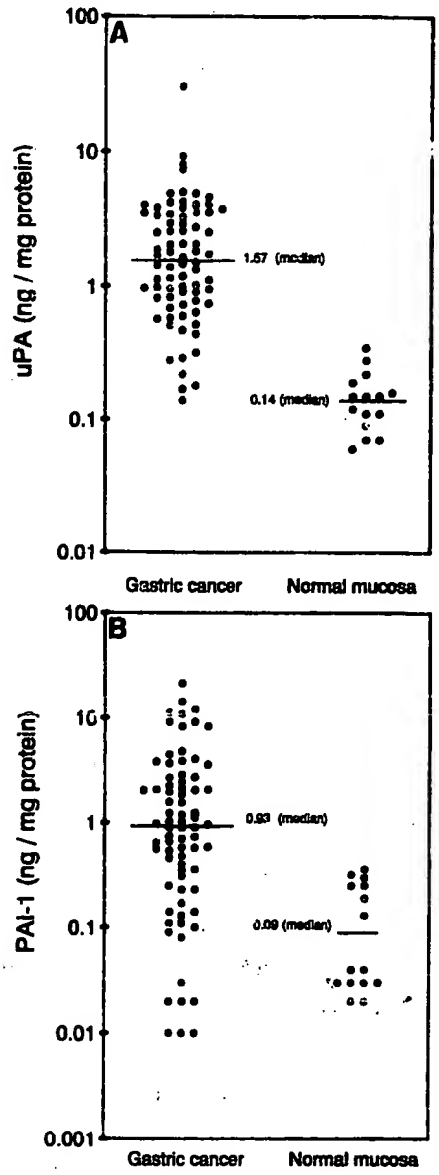

Fig. 2. uPA and PAI-1 levels in extracts of completely resected (R_0) gastric cancer tissues (n = 76) compared to extracts of normal mucosa (n = 15). uPA (*A*) and PAI-1 (*B*) values were quantified by ELISA. The data are plotted on a logarithmic scale. *Horizontal bars*, median values. Relationship between characteristics of gastric cancer and uPA and PAI-1 values are presented in Tables 1 and 2.

RESULTS

uPA and PAI-1 Levels in Gastric Cancer and Normal Mucosa. Cryostat sections prepared from 76 primary tumor specimens of completely resected gastric cancer patients and 15 normal mucosa were extracted by the nonionic detergent Triton X-100, centrifuged, aliquoted, and then analyzed for protein, uPA, and PAI-1 levels. Per tissue block, 20–30 cryostat sections (60- μm thickness; 70–100 mg wet weight) were obtained, yielding ~350 μl extract after centrifugation. The median uPA concentration of the tumor tissue extracts was 5.9 ng/ml (range, 0.38–100 ng/ml), and the median PAI-1 concentration was 4.28 ng/ml (range, 0.04–85.21 ng/ml). There is a weak correlation (r = 0.46) between tumor uPA and PAI-1 (Fig. 1). The median protein concentration of the tumor tissue extracts was 4.4 mg/ml (range, 1.19–8.52 mg/ml). The uPA and PAI-1 concentration (ng/ml) of each tumor tissue extract was divided by its respective protein concentration (mg/ml), which resulted in uPA and PAI-1 levels expressed in units ng/mg protein. A median value of 1.57 ng/mg protein (range, 0.14–30.2) for uPA and of 0.93 ng/mg protein (range, 0.01–21.2) for PAI-1 was determined (Fig. 2). In normal gastric mucosa, significantly (P < 0.001) lower levels were deter-

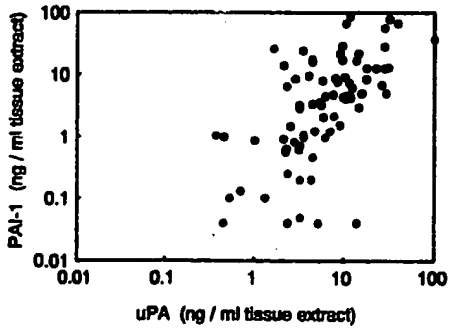

Fig. 1. Correlation of uPA and PAI-1 antigen levels determined in tumor tissue extracts by ELISA (ng/ml). Both axes are plotted logarithmically. A weak linear correlation of r = 0.46 between uPA and PAI-1 (P = 0.001) is obtained.

Table 1 *Correlation of median uPA and PAI-1 levels determined in primary tumors of completely resected (R_0) gastric cancer patients (n = 76) with criteria of the UICC staging system (TNM) and lymph node involvement*

Variable	Patients No.	Patients %	uPA (ng/mg protein)	P^a	PAI-1 (ng/mg protein)	P^a
Primary tumor (T)						
T_1	8	11	0.9 (0.1–7.4)[b]		0.1 (0.03–2.4)[b]	
T_2	42	55	1.5 (0.2–30)		1.0 (0.01–21)	
T_3	19	25	1.8 (0.2–8)		0.7 (0.01–11)	
T_4	7	9	3.4 (0.2–4.9)	n.s.	4.5 (0.01–12)	0.006[c]
Nodal status (N)						
N_0	26	34	1.0 (0.1–7.4)		0.5 (0.04–9.1)	
N_1	15	20	1.6 (0.2–8)		1.0 (0.01–21)	
N_2	35	46	2.0 (0.2–30)	n.s.	1.6 (1.1–14)	0.04[d]
Distant metastasis (M)						
M_0	65	86	1.4 (0.1–30)		0.9 (0.01–21)	
M_1 (Lym)	11	14	2.6 (0.7–4.9)	n.s.	2.0 (0.1–12)	0.05
Involvement of lymph node compartments[e]						
None	26	34	1.1 (0.1–7.4)		0.5 (0.01–9.1)	
Compartment I	28	37	1.7 (0.2–30)		1.0 (0.04–21)	
Compartment II	12	16	2.7 (0.3–9.3)		1.3 (0.2–14)	
Compartment III	10	13	2.3 (0.7–4.9)	n.s.	1.8 (0.1–12)	0.04[d]
No. of involved lymph nodes						
0	26	34	1.1 (0.1–7.4)		0.5 (0.01–9.1)	
1–3	16	21	2.2 (0.5–4.9)		1.0 (0.04–21)	
>3	34	45	2.0 (0.2–30)	n.s.	1.6 (0.02–14)	0.046[f]
Percentage of involved lymph nodes						
0	26	34	1.1 (0.1–7.4)		0.5 (0.01–9.1)	
1–20	27	36	0.8 (0.2–30)		1.0 (0.04–21)	
>20	23	30	2.0 (0.3–9)	n.s.	1.6 (0.1–14)	0.046[g]
Stages						
IA	6	8	1.0 (0.1–7.4)		0.1 (0.01–0.3)	
IB	17	22	1.1 (0.2–5)		0.7 (0.01–9)	
II	13	17	1.6 (0.6–4.9)		1.0 (1.1–21)	
IIIA	18	24	2.2 (0.2–30)		1.0 (0.01–14)	
IIIB	10	13	1.9 (0.3–3.6)		1.4 (0.2–11)	
IV	12	16	2.3 (0.2–4.9)	n.s.	1.8 (0.01–12)	0.03[h]

[a] Kruskal-Wallis group test, $P < 0.05$; Mann-Whitney two-sample test; n.s., not significant.
[b] Median value (range).
[c] T_1/T_2, $P < 0.01$.
[d] N_0/N_2, $P = 0.01$.
[e] Number according to the Japanese Research Society of Gastric Cancer.
[f] 0/>3 lymph nodes, $P = 0.01$.
[g] 0%/>20%, $P = 0.01$.
[h] IA/IB–IV, $P \leq 0.01$.

mined for uPA (0.14 ng/mg protein; range, 0.06–0.35) and for PAI-1 (0.09 ng/mg protein; range, 0.02–0.36). uPA and PAI-1 are not correlated to the extent of inflammation in the specimens investigated.

Association of Tumor uPA and PAI-1 Levels with Histomorphological Parameters. Tumor tissue uPA and PAI-1 values were compared to established histomorphological parameters (Tables 1 and 2). In contrast to uPA, PAI-1 level is significantly correlated with tumor invasion, nodal status, metastasis (TNM) and stage. Significantly different values were observed in the following categories: T_1 *versus* T_{2-4}, N_0 *versus* N_2, M_0 *versus* M_1(lym), and stage IA *versus* stages IB-IV. PAI-1 levels in primary tumor tissue extracts are significantly correlated with advanced lymph node spread (involvement of compartment III, >3 lymph nodes involved, or >20% lymph nodes involved). A lower median level of uPA (1.4 ng/mg protein) was determined in primary tumor extracts of patients without distant lymph node metastasis (M_0), in contrast to those patients having distant lymph node metastases (median value, 2.6 ng uPA/mg protein); the difference approached statistical significance ($P = 0.05$).

Additional histomorphological data related to uPA and PAI-1 are presented in Table 2. In general, primary gastric tumors that developed in the distal third of the stomach have lower median values of uPA and PAI-1 than tumors arising in other regions of the stomach. Statistically, only the PAI-1 level is significantly elevated in tumors located at the proximal third of the stomach or in tumors covering the entire stomach compared to the distal ones. PAI-1 level but not uPA level is weakly positively correlated ($P = 0.04$) with tumor size. In three tumors (4%), vascular (venous) invasion was demonstrated. These three cases were significantly associated with a relatively high level of uPA (median value, 7.4 ng uPA/mg protein). If carcinomas are subjected to the Laurén classification, significant lower median values of uPA and PAI-1 were observed for diffuse-type carcinomas than for intestinal-type carcinomas. uPA and PAI-1 are also significantly lower in signet-ring cell carcinomas (WHO classification) than in papillary differentiated and undifferentiated (medullary) carcinomas. In addition, tubulary differentiated carcinomas exhibit significantly lower median values of both uPA and PAI-1 than undifferentiated carcinomas. If tubular differentiated carcinomas are compared to papillary carcinomas, only the median uPA levels are significantly lower. Carcinomas identified macroscopically as a scirrhus-infiltrative growing pattern (Borrmann type IV) are associated with significantly lower PAI-1 values than exophytically growing carcinomas (Borrmann type I). In addition, macroscopically diagnosed early gastric carcinomas contain significantly lower levels of uPA and PAI-1 than macroscopically advanced carcinomas. Neither lymphangiosis nor perineural invasion or grading are correlated with uPA or PAI-1

Table 2 *Correlation of median levels of uPA and PAI-1 determined in primary tumors of completely resected (R_0) gastric cancer patients (n = 76) with histomorphological criteria*

Variable	Patients No.	Patients %	uPA (ng/mg protein)	P^a	PAI-1 (ng/mg protein)	P^a
Tumor site						
Proximal third	35	46	1.7 (0.3–30)[b]		1.0 (0.1–21)[b]	
Middle third	12	16	2.2 (0.1–8.1)		1.2 (0.01–11)	
Distal third	22	29	0.9 (0.2–7.4)		0.3 (0.1–14)	
Entire stomach	7	9	3.3 (1–4.1)	n.s.	2.1 (0.6–12)	0.01[c]
Grading						
1	1	1	0.4		0.1	
2	23	30	1.9 (0.3–7–4)		1.9 (0.01–21)	
3	47	62	1.5 (0.2–30)		0.7 (0.01–14)	
4	5	7	0.8 (01–2.1)	n.s.	0.4 (2.7–2.4)	n.s.
Bormann classification						
Early cancer	6	8	1 (0.1–7.1)		0.02 (0–01–0.3)	
Exophytic (I)	11	14	1.7 (0.5–4)		1.6 (0.1–11)	
Ulcer-expansive (II)	21	28	2.1 (0.6–5)		1.1 (0.1–21)	
Ulcer-infiltrative (III)	29	38	1.8 (0.2–30)		1.0 (0.04–14)	
Scirrhus-infiltrative (IV)	9	12	0.8 (0.2–3.5)	n.s.	0.4 (0.04–2.7)	0.003[d,e]
Lauren classification						
Intestinal type	44	58	2.2 (0.2–30)		1.4 (0.01–21)	
Mixed type	11	14	1.5 (0.1–9.3)		0.3 (0.01–9.1)	
Diffuse type	21	28	0.9 (0.2–3.3)	0.006[f]	0.5 (0.01–14)	0.02[g]
WHO classification						
Tubular differentiation	31	41	1.2 (0.1–4.9)		0.9 (0.01–11)	
Papillar differentiation	9	12	3.0 (0.6–5)		1.9 (0.6–21)	
Mucinous differentiation	4	5	2.6 (1–7.4)		1.3 (0.01–4.8)	
Signet-ring cells	18	24	0.9 (0.2–2.9)		0.4 (0.01–14)	
Undifferentiated	14	18	3.7 (0.7–30)	0.001[h,i]	1.8 (0.1–12)	0.02[j,k]
Perineuralcarcinosis						
No	69	91	1.5 (0.2–30)		0.9 (0.01–21)	
Yes	7	9	3.8 (0.14–4.9)		1.0 (2.7–8.3)	n.s.
Lymphangiosis carcinomatosa						
No	49	64	1.1 (0.1–30)		0.9 (0.01–21)	
Yes	27	36	2.1 (0.2–9.3)	n.s.	1.3 (0.01–12)	n.s.
Vascular invasion						
No	73	96	1.5 (0.1–30)		1.0 (0.01–21)	
Yes	3	4	7.4 (2.1–9.3)	0.03	0.9 (0.04–9)	n.s.

[a] Kruskal-Wallis group test, $P < 0.05$ and Mann-Whitney two-sample test; n.s., not significant.
[b] Median value (range).
[c] Distal/proximal, entire stomach, $P = 0.01$.
[d] Early cancer/Bormann I–IV, $P \leq 0.04$.
[e] Bormann IV/II, $P = 0.03$.
[f] Diffuse/intestinal type, $P = 0.01$.
[g] Diffuse/intestinal type, $P = 0.008$.
[h] Undifferentiated/signet-ring cell, tubular, $P = 0.004$.
[i] Papillary/signet-ring cell, tubular, $P = 0.05$.
[j] Undifferentiated/signet-ring cell, tubular, $P = 0.003$.
[k] Papillary/signet-ring cell, $P = 0.004$.

levels. No statistically significant difference is seen between protein values and number of tumor cells in the specimens.

uPA/PAI-1 and Survival Analysis. To determine whether u-PA and/or PAI-1 levels in primary tumors are of prognostic relevance in completely resected gastric cancer patients, survival was calculated using the Cox proportional hazard model. For this purpose, the observed relative risk of failure was plotted as a function of uPA and PAI-1 levels (Fig. 3). Protein (1.5 ng/mg; confidence interval, 1.33–1.73) was determined as the optimal cutoff value for uPA discriminating 37 patients (49%) with uPA <1.5 ng/mg protein and 39 patients (51%) with uPA ≥ 1.5 ng/mg protein. For PAI-1, a cutoff value of 1.25 ng/mg protein (confidence interval, 1.02–1.99) was determined, discriminating 45 patients (59%) with PAI-1 <1.25 ng/mg protein and 31 patients (41%) with PAI-1 ≥ 1.25 ng/mg protein. Actuarial survival curves (Kaplan-Meier) illustrate the increased hazard rate of patients with either high uPA or PAI-1 levels (Figs. 4 and 5). The median survival time in each identified group did not significantly differ for uPA and PAI-1: above the cutoff value, 25 (uPA) or 22 (PAI-1) months; and below the cutoff value, 44 (uPA) or 43 (PAI-1) months, respectively. It is worth mentioning that PAI-1 survival curves diverged even further than those for uPA, exhibiting a higher log-rank test value of survival ($P = 0.001$).

Survival curves (not shown) were also plotted for TNM, stage, and other important morphological variables to determine the prognostic impact of uPA and PAI-1 for survival in important subgroups: $T_{1,2}$, $N_{1,2}$, M_0, stage III+IV, and both types of WHO classification. The cutoffs for high/low uPA and PAI-1 levels used for the subgroup analysis (Table 3) were those determined for the whole sample. If the N_1 and N_2 subgroups (15 and 35 patients, respectively) are considered separately, the prognostic impact of PAI-1 for survival remains somewhat below the significance limit (N_1, $P = 0.089$; N_2, $P = 0.09$). If N_1 and N_2 are lumped together, PAI-1 is significant at the level $P = 0.014$. PAI-1 fails to be a prognostic discriminator for survival in node-negative patients, $T_{3,4}$ patients, patients with distant lymph node metastasis, and patients with stage I+II. uPA is not as powerful as PAI-1 in subgroups; it only discriminates for survival in the $T_{1,2}$, M_0,

and WHO-differentiated subgroups. However, PAI-1 discriminates in these subgroups at an even higher level of significance. One should keep in mind that statistical trends for survival in subgroups are more difficult to detect than in the entire collective.

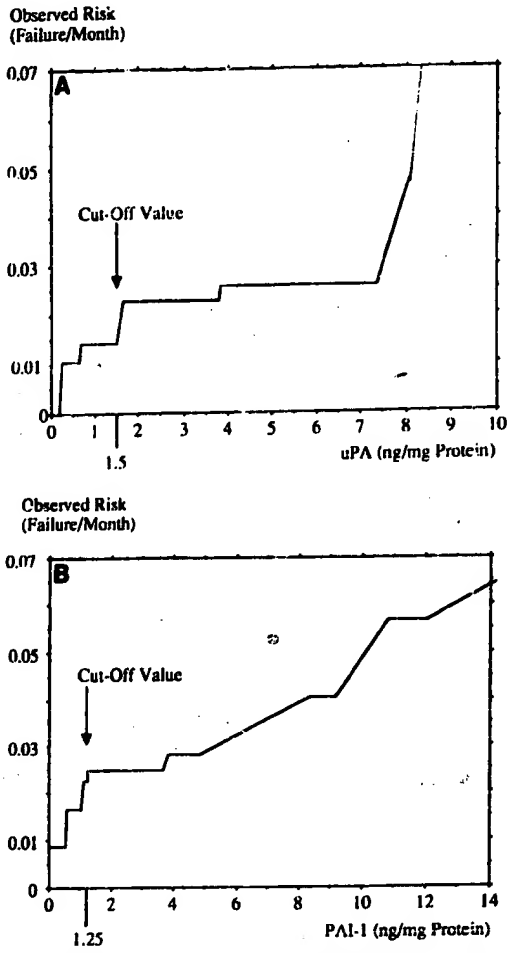

Fig. 3. Correlation of observed risk (failure/month) of death with level of uPA (*A*) and PAI-1 (*B*) in tumor tissue extracts of 76 completely resected gastric cancer patients (isotonic regression analysis). The optimal cutoff value for uPA was 1.5 ng/mg protein and for PAI-1, 1.25 ng/mg protein (CART analysis).

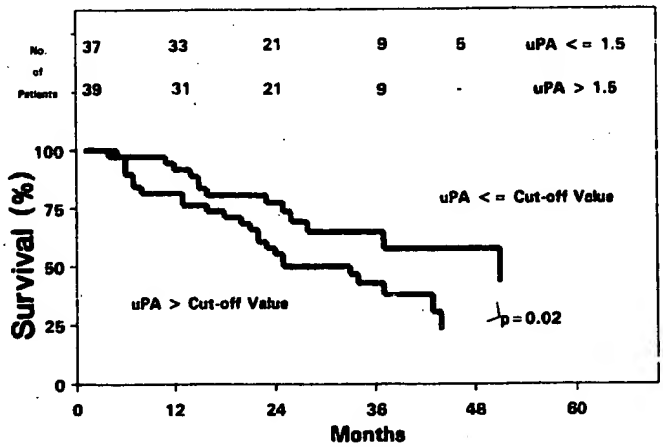

Fig. 4. Survival curve (Kaplan-Meier) computed for completely resected gastric cancer patients (n = 76) stratified by uPA level in primary tumor extracts. The cutoff value of 1.5 ng/mg protein was determined by CART analysis and isotonic regression analysis (see Fig. 3). Numbers at the top of the figure indicate patients remaining in the study at selected observation times.

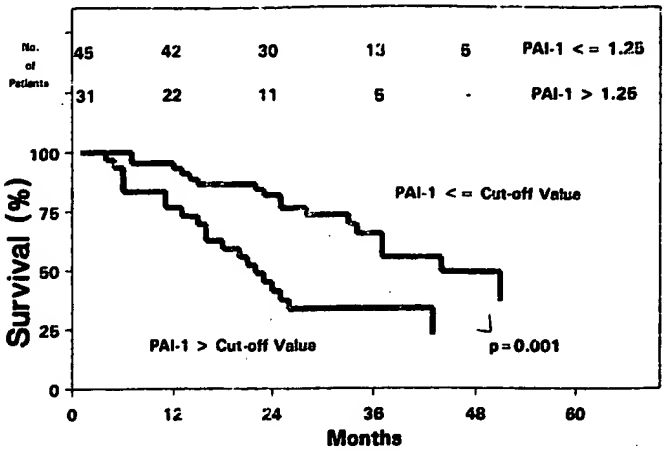

Fig. 5. Survival curve (Kaplan-Meier) computed for completely resected gastric cancer patients (n = 76) stratified by PAI-1 level in primary tumor extracts. The cutoff value of 1.25 ng/mg protein was determined by CART analysis and isotonic regression analysis (see Fig. 3). Numbers at the top as in Fig. 4.

Uni- and Multivariate Cox Regression. In univariate Cox regression analysis, TNM categories, WHO classification, and tumor size as well as uPA and PAI-1 levels are significantly correlated with survival (Table 4). In contrast, sex, site of tumor, Borrmann classification, Laurén classification, and grading are not associated with survival. To determine the independent value and the RR of the significantly correlated prognostic factors, a new Cox model (multivariate analysis) was performed (Table 5). Three prognostic factors were found to be of independent value: Nodal status, WHO classification, and PAI-1. The RR for death is five times higher in the 50 (66%) lymph node-positive patients than in the 26 node-negative patients (34%). The RR is about three times higher for the 31 patients (41%) with PAI-1 $\geq$ 1.25 ng/mg protein than for patients with lower PAI-1 levels. WHO-classification also exhibits an independent prognostic impact. The RR for death is 2.4 times higher in the 32 patients (42%) with undifferentiated carcinoma than in the 44 patients (58%) with differentiated carcinoma. Although significant in univariate analysis, the prognostic impacts of depth of invasion (T) of the primary tumor, distant lymph node metastasis [M_1(Lym)], and uPA levels were no longer significant in this model. Note however that uPA becomes an independent factor when PAI-1 is removed from the Cox model.

DISCUSSION

As yet, no tumor biological prognostic factor for increased mortality has been described in completely (R_0, UICC, 1987) resected gastric cancer. We now discuss the clinical relevance of the tumor-associated protease uPA and its inhibitor PAI-1, which are significantly elevated in gastric cancer tissue extracts compared to normal mucosa. Our new finding is that these proteolytic factors may serve as prognostic factors for overall survival in completely resected gastric cancer patients; patients with high uPA or PAI-1 antigen levels in their primary tumors exhibit shorter survival.

Our data are consistent with observations by Nishino *et al.* (14), Sier *et al.* (15), Harvey *et al.* (20), and Chung *et al.* (42), who also reported elevated uPA in gastric cancer tissue than in normal mucosa. Nakamura *et al.* (16) and Tanaka *et al.* (18) described similar findings for the inhibitor PAI-1. Takai *et al.* (17) and Harvey *et al.* (20) used 0.25% Triton X-100 for extraction; Sier *et al.* (15), Umehara *et al.* (19), and Harvey *et al.* (20) used ELISAs for uPA and PAI-1 quantification. In the other relevant references cited here, activity tests were applied. In contrast to activity tests in which only free, enzymatically active uPA can be determined, uPA-ELISAs may recognize

Table 3 *Survival analysis (log-rank test) of subgroups of completely resected gastric cancer patients (n = 76) for high or low risk determined by cutoff values of uPA and PAI-1*

Subgroup	Patients		uPA (1.5 ng/mg protein[a])		PAI-1 (1.25 ng/mg protein[a])	
	No.	%	low[b]/high[b]	P	low[b]/high[b]	P
$T_{1,2}$	50	66	28/22	0.049	32/18	0.003
$T_{3,4}$	26	34	9/17	n.s.	13/13	n.s.
N_0	26	34	18/8	n.s.	19/7	n.s.
$N_{1,2}$	50	66	19/31	n.s.	26/24	0.014
M_0	65	86	35/30	0.048	42/23	0.006
M_1	11	14	2/9	n.s.	3/8	n.s.
WHO, differentiated type	44	58	21/23	0.03	25/19	0.02
WHO, undifferentiated type	32	42	16/16	n.s.	20/12	0.007
Stage I, II	36	47	23/13	n.s.	26/10	n.s.
Stage III,IV	40	57	14/26	n.s.	19/21	0.05

[a] Cutoff value determined in the entire group of patients ($n = 76$); n.s., $P > 0.05$.
[b] Number of grouped patients.

Table 4 *Relative risk of survival assessed by univariate and multivariate cox regression analysis in completely resected gastric cancer patients (n = 76)*

Variable	Category	Univariate analysis P*	Multivariate Analysis P*	Relative risk	Confidence interval
Primary tumor	$pT_{1,2}/T_{3,4}$	0.0004	n.s.		
Nodal status	$pN_0/N_{1,2}$	<0.0001	0.004	5.04	1.74–14.59
Distant metastasis	pM_0/M_1 (Lym)	0.0004	n.s.		
WHO classification	Differentiated/undifferentiated	0.015	0.014	2.37	1.19–4.7
PAI-1 (ng/mg protein)	≤1.25/>1.25	0.001	0.0016	2.94	1.5–5.78
uPA (ng/mg protein)	≤1.5/>1.5	0.009	n.s.		

[a] n.s., $P > 0.05$.

various forms of uPA, including the enzymatic inactive precursor pro-uPA, free and complexed forms of the activated, two-chain form of uPA, and (pro)-uPA bound to its receptor uPA-R (10, 12, 35). Likewise, various inactive and active forms of PAI-1 are recognized by the PAI-1-ELISA used (10). ELISA tests for uPA and PAI-1 have been used previously in prognosis-related breast cancer studies and were found to be superior to activity assays (9–13).

In our group of completely resected gastric cancer patients, various histomorphological parameters are related to uPA and PAI-1 levels. A significantly lower level of uPA and PAI-1 was observed in signet-ring cell carcinomas, tubular-differentiated carcinomas (WHO classification), and diffuse-type carcinomas (Laurén classification). Increased uPA and PAI-1 antigen was determined in papillary carcinomas, poorly differentiated carcinomas (WHO classification), and intestinal-type carcinomas (Laurén classification).

Reports concerning levels of enzymatically active tumor-associated uPA and its inhibitor PAI-1 in gastric cancer in relation to histomorphological classification are contradictory. One obvious explanation for such discrepancies could be nonuniform interpretation of tumor histology by different investigators (for a review, see Ref. 43), especially the WHO classification (33, 44–46). Similarly to our findings, significantly increased uPA levels were reported by Umehara *et al.* (19) to be correlated with the undifferentiated type of gastric carcinoma; Takai *et al.* (17) reported that lower uPA values are associated with incidence of signet-ring cell carcinomas.. In the same investigation, Takai *et al.* demonstrated (in contrast to our results) significantly lower uPA values in poorly differentiated gastric cancer. In another investigation, Nakamura *et al.* (16) did not find any correlation between uPA or PAI-1 with WHO classification. Our study reveals that the WHO classification suggested by Sugano (33) is itself an independent prognostic factor for survival and that elevated levels of uPA or PAI-1 predict high-risk patients in both WHO subclasses. These findings suggest that biochemical factors, *e.g.*, tumor invasion-associated proteases, are most likely related to the individual biological behavior of a particular cancer rather than to the histomorphological architecture of the tumor.

Gabbert *et al.* (47) reported tumor-cell dissociation at the invasion front to be an independent prognostic parameter for survival in intestinal-type gastric cancer, significantly associated with vascular and lymphatic invasion. In this respect, tumor cell dissociation was proposed to be one of the first steps in the cascade of tumor invasion (2) and was suspected to be related to down-regulation or malfunction of adhesion-factors, *e.g.*, E-cadherin (48). A second, equally important step is the subsequent degradation of the extracellular matrix by tumor-associated proteases, facilitating tumor spread and metastasis (2, 4). In our study, uPA was significantly correlated with the presence of vascular invasion but not with TNM. PAI-1 was found to be correlated with advanced lymph node involvement, depth of tumor invasion, and tumor stage. This finding is consistent with two other independent studies by Nakamura *et al.* (16) and Takai *et al.* (17), but it is in conflict with the results of Umehara *et al.* (19), who showed a significant correlation between uPA and depth of invasion, nodal status, and stage.

The only survival-related independent prognostic factors in patients with completely resected (R_0, UICC) gastric cancer known up till now are histomorphological parameters such as nodal status, depth of invasion, size and site of tumor, distant metastasis, Borrmann classification, WHO classification, and presence of complications which may follow after surgery (26, 43–47, 49). None of the above references related elevated uPA and/or PAI-1 values in gastric cancer to

prognosis (survival). In our group of 76 completely resected gastric cancer patients, multivariate survival analysis (including a variety of prognostic factors determined by univariate analysis) reveals nodal status, PAI-1 levels, and WHO classification (33) to be the only three independent prognostic factors, with relative risks of 5-, 2.9-, and 2.4-fold, respectively.

PAI-1 (10, 13) has already been shown for breast cancer patients to be an independent prognostic factor for both recurrence and survival, even in node-negative patients. Although the observed risk of failure for patients with gastric cancer or for those with breast cancer (10, 12) is similarly correlated with levels of uPA and PAI-1, uPA and PAI-1 cutoffs in the breast cancer study of Jänicke *et al.* (10) differ from ours, although the very same ELISA tests and method of tissue extraction were used. Cutoffs for uPA and PAI-1 are lower in gastric cancer than in breast cancer: uPA (breast cancer, 2.97; gastric cancer, 1.5 ng/mg protein); and PAI-1 (breast cancer, 2.18; gastric cancer, 1.25 ng/mg protein). This discrepancy could be due to a difference in tumor histology, or it may merely reflect tumor biological features of the different types of cancer. Nevertheless, the prognostic impact (RR) of PAI-1 as determined by multivariate analysis ranged between 2- and 3-fold, both in breast cancer (10) and in gastric cancer specimens (this study).

We attempted to determine the prognostic impact of uPA and PAI-1 in clinically important subgroups of gastric cancer. On the basis of uPA and PAI-1 levels (with cutoffs determined from the sample as a whole), we were able to distinguish patients having worse survival within certain subgroups ($T_{1,2}$, $N_{1,2}$, M_0, stage III+IV, and WHO classification). Now, a definitive statistical analysis for survival in subgroups will require a larger number of patients. Nevertheless, our data strongly support the conclusion that the tumor-associated protease inhibitor PAI-1, and to a lesser extent uPA, should be considered as new and powerful prognostic factors for survival in completely resected gastric cancer patients.

The prognostic impact of PAI-1 (multivariate analysis) in completely resected gastric cancer is higher than that of uPA. This seems somewhat contradictory, since one would expect high levels of the inhibitor PAI-1 to act protectively by blocking the enzymatic activity of receptor-bound uPA (1–3). However, as pointed out by Jänicke *et al.* (10) in the context of breast cancer prognosis, high levels of PAI-1 may be of importance for reimplantation of circulating tumor cells at distant loci. Generation and growth of metastases require the formation of a new tumor stroma, which occurs via prevention of uPA-mediated degradation of the extracellular matrix. Moreover, since in normal tissue PAI-1 is present in endothelial cells and platelets, increased PAI-1 levels may reflect a high degree of angiogenesis, thus favoring tumor spread and metastasis. Detailed immunohistochemical and enzymological analysis will therefore be performed to localize uPA and PAI-1 in gastric cancer tissues in order to find out whether uPA concentrations are greatest in tumor cells or in accessory cells at invasive fronts, as well as whether PAI-1 concentrations are highest in main tumor bodies, thereby protecting them from damage by uPA-mediated proteolysis.

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REFERENCES

1. Danø, K., Andreasen, P. A., Grøndahl-Hansen, J., Kristensen, P., Nielsen, L. S., and Skriver, L. Plasminogen activators, tissue degradation, and cancer. Adv. Cancer Res., *44:* 139–266, 1985.
2. Hart, I. R., and Saini, A. Biology of tumor metastasis. Lancet, *339:* 1453–1457, 1992.
3. Vassalli, J-D., Sappino, A-P., and Belin, D. The plasminogen activator/plasmin system. J. Clin. Invest., *88:* 1067–1072, 1991.
4. Zacharski, L. R., Wojtukiewicz, M. Z., Constantini, V., Ornstein, D. L., and Memoli, V. A. Pathways of coagulation/fibrinolysis activation in malignancy. Semin. Thromb. Hemostasis, *18:* 104–116, 1992.
5. Liotta, L. A., Steeg, P. S., and Stetler-Stevenson, W. G. Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation. Cell, *64:* 327–336, 1991.
6. Frost, P., and Levin, B. Clinical implications of metastatic process. Lancet, *339:* 1458–1461, 1992.
7. Spyratos, F., Maudelonde, T., Brouillet, J-P., Brunet, M., Defrenne, A., Andrieu, C., Hacene, K., Desplaces, A., Rouesse, J., and Rochefort, H. Cathepsin D: an independent prognostic factor for metastasis of breast cancer. Lancet, *2:* 1115–1118, 1989.
8. Tandon, A. T., Clark, G. M., Chamness, G. C., Chirgwin, J. M., and McGuire, W. L. Cathepsin D and prognosis in breast cancer. N. Engl. J. Med., *322:* 297–302, 1990.
9. Jänicke, F., Schmitt, M., Hafter, R., Hollrieder, A., Babic, R., Ulm, K., Gössner, W., and Graeff, H. Urokinase-type plasminogen activator (uPA) antigen is a predictor of early relapse in breast cancer. Fibrinolysis, *4:* 69–78, 1990.
10. Jänicke, F., Schmitt, M., Pache, L., Ulm, K., Harbeck, N., Höfler, H., and Graeff, H. Urokinase (uPA) and its inhibitor PAI-1 are strong and independent prognostic factors in node-negative breast cancer. Breast Cancer Res. Treat., *24:* 195–208, 1993.
11. Duffy, M. J., Reilly, D., O'Sullivan, C., O'Higgins, N., Fennelly, J. J., and Andreasen, P. Urokinase-plasminogen activator, a new and independent prognostic marker in breast cancer. Cancer Res., *50:* 6827–6829, 1990.
12. Foekens, J. A., Schmitt, M., van Putten, W. L. J., Peters, H. A., Bontenbal, M., Jänicke, F., and Klijn, J. G. M. Prognostic value of urokinase-type plasminogen activator in 671 primary breast cancer patients. Cancer Res., *52:* 6101–6105, 1992.
13. Grøndahl-Hansen, J., Christensen, I. J., Rosenquist, C., Brünner, N., Mouridsen, H. T., Danø, K., and Blichert-Toft, M. High levels of urokinase-type plasminogen activator and its inhibitor PAI-1 in cytosolic extracts of breast carcinomas are associated with poor prognosis. Cancer Res., *53:* 2513–2521, 1993.
14. Nishino, N., Aoki, K., Tokura, Y., Sakaguchi, S., Takada, Y., and Takada, A. The urokinase type of plasminogen activator in cancer of digestive tracts. Thromb. Res., *50:* 527–535, 1988.
15. Sier, C. F. M., Verspaget, H. W., Griffioen, G., Ganesh, S., Vloedgraven, H. J. M., and Lamers, C. B. H. Plasminogen activators in normal tissue and carcinomas of the human oesophagus and stomach. Gut, *34:* 80–85, 1993.
16. Nakamura, M., Konno, H., Tanaka, T., Maruo Y., Nishino, N., Aoki, K., Baba, S., Sakaguchi, S., Takada, Y., and Takada, A. Possible role of plasminogen activator inhibitor 2 in the prevention of the metastasis of gastric cancer tissues. Thromb. Res., *65:* 709–719, 1992.
17. Takai, S., Yamamura, M., Tanaka, K., Kawanishi, H., Tsuji, M., Nakane, Y., Hioki, K., and Yamamoto, M. Plasminogen activators in human gastric cancers: correlation with DNA ploidy and immunhistochemical staining. Int. J. Cancer, *48:* 20–27, 1991.
18. Tanaka, N., Fukao, H., Ueshima, S., Okada, K., Yasutomi, M., and Matsuo, O. Plasminogen activator inhibitor 1 in human carcinoma tissues. Int. J. Cancer, *48:* 481–484, 1991.
19. Umehara, Y., Kimura, T., Yoshida, M., Oka, N., and Harada, Y. Relationship between plasminogen activators and stomach carcinoma stage. Acta Oncol., *30:* 815–818, 1991.
20. Harvey, S. R., Lawrence, D. D., Madeja, J. M., Abbey, S. J., and Markus, G. Secretion of plasminogen activators by human colorectal and gastric tumor explants. Clin. Exp. Metastasis, *6:* 431–450, 1988.
21. De Bruin, P. A. F., Griffioen, G., Verspaget, H. W., Verheijen, J. H., Dooijewaard, G., van den Ingh, H. F., and Lamers, C. B. H. W. Plasminogen activator profiles in neoplastic tissues of the human colon. Cancer Res., *48:* 4520–4524, 1988.
22. Sim, P. S., Stephens, R. W., Fayle, D. R., and Doe, W. F. Urokinase-type plasminogen activator in colorectal carcinomas and adenomatous polyps: quantitative expression of active proenzyme. Int. J. Cancer, *42:* 483–488, 1988.
23. Gelister, J. S. K., Mahmoud, M., Lewin, M. R., Gaffney, P. J., and Boulos, P. B. Plasminogen activators in human colorectal neoplasia. Br. Med. J., *293:* 728–731, 1986.
24. Sier, C. F. M., Fellbaum, C., Verspaget, H. W., Schmitt, M., Griffioen, G., Graeff, H., Höfler, H., and Lamers, C. B. H. W. Immunolocalization of urokinase-type plasminogen activator in adenomas and carcinomas of the colorectum. Histopathology (Oxf.), *19:* 231–237, 1991.
25. Union International Contre Cancer. TNM-Classification of Malignant Tumors, Ed. 4. Berlin: Springer-Verlag, 1987.
26. Roder, J. D., Böttcher, K., Siewert, J. R., Busch, R., Hermanek, P., Meyer, H. J., and the German Gastric Carcinoma Study Group. Prognostic factors in gastric carcinoma. Results of the German Gastric Carcinoma Study 1992. Cancer (Phila.), *72:* 2089–2097, 1993.
27. Siewert, J. R., Böttcher, K., Roder, J. D., Busch, R., Hermanek, P., Meyer, H. J., and the German Gastric Carcinoma Study Group. Prognostic relevance of systematic lymph node dissection in gastric carcinoma. Br. J. Surg., *80:* 1015–1018, 1993.
28. Japanese Research Society for Gastric Cancer. The general rules for the gastric cancer study in surgery and pathology. Jpn. J. Surg., *11:* 127–145, 1981.
29. Siewert, J. R. Lymphadenektomie beim Magenkarzinom. Chirurg, *62:* 881–884, 1991.
30. Borrmann, R. Geschwülste des Magens und Duodenums. *In:* F. Henke and O. Lubarsch, (eds.). Handbuch der speziellen pathologischen Anatomie und Histologie, Vol. 4., p. 865. Berlin: Springer-Verlag, 1926.
31. Oota, K., and Sobin, L. H. Histological typing of gastric and esophageal tumours. *In:* International Histological Classification of Tumours, No. 18, Geneva: WHO, 1977.

32. Lauren, P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. Acta Path. Microbiol. Scand., *64*: 31–49, 1965.
33. Sugano, H., Nakamura, K., and Kato, Y. Pathological studies of human gastric cancer. Acta Pathol. Jpn., *32*: 329–347, 1982.
34. Kaplan, E. L., and Meier, P. Nonparametric estimation from incomplete observations. J. Am. Stat. Assoc., *53*: 457–481, 1958.
35. Schmitt, M., Janicke, F., and Graeff, H. Tumor-associated fibrinolysis: the prognostic relevance of plasminogen activators uPA and tPA in human breast cancer. Blood Coagul. Fibrinolysis, *1*: 695–702, 1990.
36. Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J., and Klenk, D. C. Measurement of protein using bicinchoninic acid. Anal. Biochem., *150*: 76–85, 1985.
37. BMDP Statistical Software. Berkeley, CA: University of California Press, 1985.
38. Cox, D. R. Regression models and life-tables. J. R. Stat. Soc., *34*: 187–220, 197?
39. Breimann, L., Friedmann, J. H., Ohlsen, R. A. and Stone, C. J. Classification a. Regression Trees (CART). Belmont, CA: Wadsworth International Group, 1984.
40. Gordon, L., and Ohlsen, R. A. Tree-structured survival analysis. Cancer Treat. Rep., *69*: 1065–1069, 1985.
41. Segal, M. R., Regression trees for censored data. Biometrics, *44*: 35–47, 1988.
42. Chung, S. M., and Kawai, K. Protease activities in gastric cancer tissues. Clin. Chim. Acta, *189*: 205–210, 1990.
43. Devessar, K., Pezzullo, J. C., Kessimian, N., Hale, J. H., and Jauregui, H. O. Gastric adenocarcinoma: prognostic significance of several pathologic parameters and histological classifications. Hum. Pathol., *21*: 325–332, 1990.
44. Maruyama, K. J. The most important prognostic factors for gastric cancer patients. A study using univariate and multivariate analyses. Scand. J. Gastroenterol., *22*: 63–68, 1987.
45. Koga, S., Kishimoto, H., Tanaka, K., and Kawaguchi, H. Clinical and pathological evaluation of patients with recurrence of gastric cancer more than five years postoperatively. Am. J. Surg., *136*: 317–321, 1978.
46. Moriguchi, S., Kamakura, T., Odaka, T., Nose, Y., Maehara, Y., Korenaga, D., and Sugimachi, K. Clinical features of the differentiated and undifferentiated types of advanced gastric carcinoma: univariate and multivariate analyses. J. Surg. Oncol., *48*: 202–206, 1991.
47. Gabbert, H. E., Meier, S., Gerharz, C. D., and Hommel, G. Tumor-cell dissociation at the invasion front: a new prognostic parameter in gastric cancer patients. Int. J. Cancer, *50*: 202–207, 1992.
48. Becker, K. F., Atkinson, M. J., Reich, U., Huang, H. H., Nekarda, H., Siewert, J. R., and Höfler, H. Exon skipping in the E-cadherin gene transcript in metastatic human gastric carcinomas. Hum. Mol. Genet., *2*: 803–804, 1993.
49. Nakamura, K., Ueyama, T., Yao, T., Xuan, Z. X., Ambe, K., Adachi, Y., Yakeishi, Y., Matsukuma, A., and Enjoji, M. Pathology and prognosis of gastric carcinoma. Findings in 10,000 patients who underwent primary gastrectomy. Cancer (Phila.), *70*: 1030–1037, 1992.